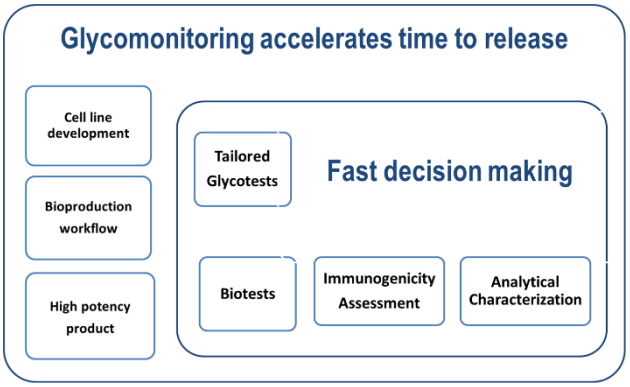
Glycomonitoring: a fast decision making for high potency biologics

Glycomonitoring addresses 7 key steps to be considered very closely when manufacturing a human protein which is glycosylated. Glycosylation of a lead candidate is protein-, cell- and process dependent. The protein glycoprofile therefore contains a variable bar code which may be tracked at all steps to improve product quality and satisfy regulatory compliance.

So far however, it has been challenging to predict, achieve and maintain preferred glycosylation profiles from clone generation to bioproduction. This is not merely incidental but proved to be crucial for the safety and efficacy of most biologics. To deliver high potency biologics, there is a clear need to select drug candidates with the most appropriate glycoprofile. Thorough investigation at the earliest stage will avoid delays during late stage development.



1. **Customization of glycosylation :** glycosylation appears as the most challenging critical quality attribute because of its variability. This impacts on lifespan, immunogenicity and biopotency of therapeutics. Biosimilars require a glycoprofile comparable if not identical to the originators, serum glycoproteins (cytokines, growth factors, enzymes..) may require multiantennary glycans to be optimally active and antibodies generally show a truncated glycosylation naturally occurring in inflammatory diseases. In all cases, glycosylation contains a complex sugar barcode which allows a protein to bind biological target(s) and immune cells simultaneously, clearing some glycoforms and signaling others to immune recognition within minutes. To deliver long lasting and safe products, it is therefore crucial to identify the most appropriate glycoprofile for each product from the start, govern and control its quality during all bioprocesses. Glycomonitoring provides a fast-track method to achieve this characterization.

2. **Choice of the cell line** : Biologics can receive a quite distinct glycosylation pattern when expressed in insect, plant, mammalian or human cell lines. In all cases, the presence of antigenic sugars should be tested in N-linked glycans as well as in O-linked glycans and avoided. Today, engineering CHO cell lines has delivered robust cell lines to reach high density and produce high titers of the protein of interest. However, glycosylation of both cells and product remains highly variable upon cultivation. Glycotests can monitor the glycosylation status of the choosen cell line to maximize its capacity and achieve the preferred glycoprofile for the product.

3. **Developability assessment :** Methods to monitor most quality attributes have been established and product glycosylation is the most critical as glycan analysis requires release and analysis remotely from production. Glycomonitoring is a new analytical strategy to identify rapidly the best glycoprofile at line and obtain the desired biological performances for the product. Customizable glycotests allow screening of a wide array of

experimental conditions to select the most promising samples to be further characterized . They facilitate decision making on line as well as the developability of a lead candidate.

4. **Cell line development** : To create robust manufacturing processes, high producing cell lines are generally selected for displaying the highest productivity together with the most suitable growth. This definitely impacts the glycosylation machinery of the selected clones which may or may not synthesize the desired glycoprofile . Glycomonitoring helps identifying high producing clones which display both the highest quality and the highest metabolic capacity. Based on lectin assays, it applies to all families of biologics. Glycotests focus on key sugar(s) of special biological interest, most often mannose, fucose and sialic acid.

5. **Early development process :** Clone optimization is a lengthy and hazardous process which assesses comparability for biosimilars or early developability for new molecules to maximize bioproduction. Post translational modifications of the product are generally analyzed at the final steps. Glycomonitoring offers customizable glycotests which can be run by hundreds within hours. Changes in product glycoprofile are rapidly identified along with Quality by Design experiments. This screening helps improving early formulability and stability of the product. In addition, early immunogenicity assessment may also be introduced to prevent potential adverse effects in clinics. This is of special importance for biosimilars for which immunogenicity of the originators has been largely documented. In the context of new protein constructs, identifying which glycoprofile is best tolerated may prevent the hurdle of regulatory compliance and accelerate time to release.

6. **Process development:** To keep the product at the highest biological performance throughout the overall process development, the glycoprofile identified as optimal in earlier steps should be reproduced throughout the bioproduction workflow. Glycomonitoring provides a creative approach to make decision with confidence and without delay to maximize process consistency. It is fully complementary to current strategies and further aims at accelerating development timelines.

7. **Analytical development** : Throughout manufacturing, we need methods that delivers reliable results, makes effective use of analytics and are easy to scale up across a range of processes: using glycosylation as a barcode of product quality helps analytics to focus on the best achievable glycoprofile to be extensively characterized. Glycomonitoring is fully complementary to high-throughput, automation, QbD and other strategies to determine developability, stability and early formulability of the molecules and produce high potency biologics.

Summary :

From lead identification to full scale bioproduction, regulators are requesting deeper knowledge about the process and products : using glycosylation as a bar code for product quality will facilitate the overall production workflow for new candidates.

Glycomonitoring is a fast decision making for accelerating development timelines and producing high potency biologics.

Information : catherine.ronin@siamed-tech.com